ELECTRONIC SOMATIC CELL COUNT
Foss 90

(Unless otherwise stated all tolerances ±5%)

1. Laboratory Requirements (see CP, item 33 & 34) _____

   a. Un-preserved samples may be run from 24 to 72 hours after initial collection _____

   b. Samples may be run from 8 hours up to 7 days after initial collection if preserved with 0.02% 2-bromo-2-nitropropane-1,3-diol (Bronopol™)or 0.05% potassium dichromate ($K_2Cr_2O_7$) _____

   c. Comparative test with DMSCC _____

      1. Performed by each analyst performing ESCC test _____

      2. Test 4 samples (100K-200K, 300K-500K, 600K-800K and 900K-1.2M) in triplicate for both DMSCC (three separate smears each) and ESCC (three separate sub-samples each, do not read same sample three times) _____

      3. Results must be shown to be acceptable prior to official testing by analyst performing comparison, i.e. analyst is not certified until found acceptable. **(co-requisite for certification)** _____

      4. Copy of comparison and results in QC record (or easily accessible file in laboratory) _____

   d. Analysts certified in DMSCC _____

APPARATUS

2. See Cultural Procedures, items 1 - 5 _____

3. Fossmatic 90 Electronic Somatic Cell Counter _____

4. Pipettor, fixed volume or electronic (_____) _____

   a. Calibrated to deliver 500 µL milk (see CP item 6e) _____

   b. Records maintained _____

5. Pipettor Tips _____

   a. Disposable, replace for each sample _____

   b. Reusable _____

      1. Rinse in 40C deionized (DI) or MS water _____

      2. Rinse in sample more than 1 time _____

3. Do not use for more than 25 samples _____

6. Water Bath _____

a. Circulating and thermostatically controlled to 37-42C _____

REAGENTS

7. Stock Dye Solution, 0.1% Ethidium Bromide (caution TOXIC, use gloves when handling and do not breath dust) _____

a. Dissolve 1.0g ethidium bromide ($C_{21}H_{20}BrN_3$) in 1 liter DI or MS water by heating to 40-60C _____

b. Store in light-proof, air-tight bottle no more than 60 days _____

c. Date prep. _____ Exp. Date _____ _____

8. Stock Rinsing Solution, 1% Triton X-100 _____

a. Dissolve 10 mL Triton X-100 in 1 liter DI or MS water by heating to 60C _____

b. Store in air-tight container no more than 25 days _____

c. Date prep. _____ Exp. Date _____ _____

9. Stock Buffer Solution, 0.025 M Potassium Hydrogen Phthalate _____

a. Dissolve 51.0g KH phthalate and 13.75g KOH in 10 L DI or MS water by heating to 40-60C _____

b. Add 150 mL 1% Triton X-100 (item 8), store less than 7 days in airtight container _____

c. Date prep. _____ Exp. Date _____ _____

10. Ammonium Hydroxide (NH_4OH) Solution, Reagent Grade, 25% _____

11. All stock dye/buffer/rinsing solutions labeled with date prepared and expiration date _____

WORKING SOLUTIONS

12. Working Dye Solution/Zero Control (used within 7 days) _____

a. Dilute 26 mL stock dye solution (item 7a) to 2.5 liter with stock buffer solution (item 9b2) _____

b. Date prep. _____ Exp. Date _____ _____

13. Working Rinsing Solution (used within 7 days) _____

a. Add 10 mL stock rinsing solution (item 8) to 25 mL of 25% NH_4OH and dilute to 10 liters with DI or MS water _____

b. Date prep. _____ Exp. Date _____ _____

14. Optionally use manufacturer's reagent kits and instructions specific for each instrument _____

15. All working dye and rinsing solutions labeled with date prepared and expiration date _____

START UP

16. Cell Counter _____

    a. Assure adequate volume of working solutions, not used beyond expiration date(s) _____

    b. Turn on power and cycle at least six times _____

    c. Blind count Ω5 _____

    d. Vacuum pressure setting minimum of -40 KPa _____

    e. Dispenser filling time 4-5 seconds _____

    f. Intake filling time 3-4 seconds _____

    g. **IF ANY ABOVE PARAMETERS ARE WRONG, CORRECT BEFORE PROCEEDING** _____

    h. Records maintained on all parameters _____

17. Milk Standards _____

    a. Commercially prepared: _____
    Lot# _____ Date Rcd. _____ _____

        1. Four samples in ranges 100K-200K, 300K-500K, 600K-800K and 900K-1.2M _____

        2. Do DMSCC in triplicate on each standard in set and average counts, records maintained _____

        3. DMSCC check performed in rotation by all certified analysts _____

        4. Standards used within one week _____

    b. Certified provider: _____
    Lot# _____ Exp. Date _____ Date Rcd. _____ _____

        1. Four standards in ranges 100K-200K, 300K-500K, 600K-800K and 900K-1.2M _____

        2. Maintain copies of all provided DMSCC values _____

        3. Measure and maintain records of temperature (0-7.2C) of standards as received _____

        4. Maintain copies of all correspondence regarding problems _____

5. Standards used by manufacturer's expiration date _____

c. Laboratory prepared (weekly) _____

1. Prepare from raw milk > 18 hours old preserved with 0.05% potassium dichromate ($K_2Cr_2O_7$) _____

2. Or, preserved with 0.02% 2-brono-2-nitropropane-1,3-diol (Bronopol™) _____

3. Standards <u>cannot</u> be preserved with formalin _____

4. Prepare 4 standards in ranges 100K-200K, 300K-500K, 600K-800K and 900K-1.2M, used within one week _____
Date prep. _____ Exp. Date _____ _____

5. Do DMSCC in triplicate on each standard prepared and average counts, records maintained _____

6. DMSCC check performed in rotation by all certified analysts _____

d. Hourly Control Sample (instrument drift check) _____

1. Use one of the standards (items 17a or b) in the 500-800K range, run in triplicate and determine average _____

2. Optionally, prepare sufficient control/sample 500-800K range, run in triplicate and determine average _____

PROCEDURE

18. Testing Standards (each time instrument used) _____

a. Heat standards to 37-42C (using a temperature control) and read within 30 minutes of reaching temperature, used once and then discarded, i.e., do not re-use _____

b. Invert 10 times, pipet 500 µL into intake chamber within 3 min _____

c. Run standards in triplicate and average the counts for each level, records maintained _____

d. Each standard's average must be within 10% of the DMSCC (item 17) for that level, except within 15% for 100-200K standard, records maintained _____

e. Repeatability - a standard in the 300K to 800K range must have a coefficient of variation (C_v) of 5% or less on 10 replicates **(Refer to Operating Manual)**, records maintained _____

f. **THESE PARAMETERS MUST BE ACHIEVED BEFORE PROCEEDING** _____

19. Testing Samples _____

    a. Heat samples to 37-42C (using a temperature control) and read within 30 minutes of reaching temperature, samples must not be re-used and must be discarded after use _____

    b. Invert 10 times, pipet 500 µL into intake chamber within 3 min _____

    c. Record number of cells counted for each sample _____

20. With continuous operation: _____

    a. Run a standard or optionally a control/sample (item 17d) in the 500K to 800K range hourly, must be within 5% of the original established instrument average value (optionally, within 10% of original DMSCC average) _____

    b. Run standard/control 3x _____

    c. Run zero control (in item 12) _____

    d. Maintain records _____

21. Routine maintenance _____

    a. Perform as described in operating manual _____

    b. Maintain records _____

REPORTS

22. Computing and Reporting Counts _____

    a. Count obtained x 1000 is the cell count/mL milk _____

    b. In reporting electronic somatic cell counts (ESCC/mL), record only first two left hand digits, raising second digit to next higher number when third digit is 6 or more _____

    c. Report the two left hand digits (rounded) _____

        1. If the third digit is 5 the second digit is rounded by the following rule _____

            a. When the second digit is odd round up, raise the second digit by 1 (odd up, 235 to 240) _____

            b. When the second digit is even round down, delete the 5 and report the second digit as is (even down, 225 to 220) _____

    d. If count on instrument is < 100 report as < 100,000 ESCC/mL _____